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Recurrent loss of heterozygosity in 1p36 associated with *TNFRSF14* mutations in *IRF4* translocation negative pediatric follicular lymphomas

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Running heads: *TNFRSF14* mutations and molecular subtypes in pedFL

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Abstract

Pediatric follicular lymphoma is a rare disease that differs genetically and clinically from its adult counterpart. With the exception of pediatric follicular lymphoma with *IRF4*-translocation, the genetic events associated with these lymphomas have not yet been defined. We applied array-comparative genomic hybridization and molecular inversion probe assay analyses to formalin-fixed paraffin-embedded tissues from 18 patients aged ≤18 years with *IRF4* translocation negative follicular lymphoma. All evaluable cases lacked t(14;18). Only six of 16 evaluable cases displayed chromosomal imbalances with gains or amplifications of 6pter-p24.3 (including *IRF4*) and deletion and copy number neutral-loss of heterozygosity in 1p36 (including *TNFRSF14*) being most frequent. Sequencing of *TNFRSF14* located in the minimal region of loss in 1p36.32 showed nine mutations in seven cases from our series. Two subsets of pediatric follicular lymphoma were delineated according to the presence of molecular alterations, one with genomic aberrations associated with higher grade and/or diffuse-large-B-cell lymphoma component and more widespread disease, and another one lacking genetic alterations associated with more limited disease.

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Introduction

Follicular Lymphoma (FL) is the most frequent indolent lymphoma in the Western World and accounts for about 20-40% of all B-cell lymphomas.[1] Pediatric FL are considered as variant of FL that differ from adult FL with an increased proportion exhibiting high grade histology and most cases presenting clinically as localized and curable diseases.[2-4] Moreover, pediatric FL differ genetically from their adult counterpart.[2] The genetic hallmark of adult FL, the translocation t(14;18)(q32;q21) affecting the *BCL2* gene is hardly ever present in pediatric FL.[2,3] Overall and in contrast to adult FL, the genetic events associated with pediatric FL pathogenesis and prognosis have not yet been defined, with the notable exception of those pediatric cases carrying an *IRF4* translocation.[4] Thus, we have here applied array-comparative genomic hybridization (aCGH) and molecular inversion probe (MIP) assay adapted for formalin-fixed, paraffin-embedded (FFPE) tissues to FL of 18 patients diagnosed up to the age of 18 years.

Methods

The study included 18 pediatric FL lacking an *IRF4* translocation with available FFPE tissue diagnosed in patients aged up to 18 years; ten cases were previously reported with regard to clinicopathological features.[3] With the exception of one case with only focal involvement by lymphoma (pFL13), the tumor cell content exceeded 50% in the evaluable samples. Eleven of 18 patients were treated according to NHL-BFM group multicenter trials,[5,6] whereas the remaining according to different treatment strategies.

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Clinical and histopathologic data are summarized in supplemental material and Supplementary Table 1.

DNA was extracted from FFPE tissue blocks using a phenol-chloroform extraction method.[7] Fourteen cases were hybridized on the MIP-assay using Oncoscan FFPE Express custom service (Affymetrix, Santa Clara, CA).[8] Thirteen cases were analyzed using Agilent 244K array (Agilent Technologies, Santa Clara, CA), including nine cases simultaneously analyzed by MIP-assay (Supplementary information and Supplementary Figure 1). Copy number (CN) plots were generated by the use of Nexus 6.0 beta Discovery Edition software (Biodiscovery, El Segundo, CA). Gains and losses were evaluated by two different observers.

Fluorescence *in situ* hybridization (FISH) analyses were performed for the detection of breakpoints or gene fusions and for verification of gains in chromosome 6p25 as previously described.[9,10] For this purpose commercially available *MYC* BAP, *BCL2* BAP, *IGH* BAP and *BCL6* BAP probes, an *IGH/BCL2* double-color double-fusion probe (all Abbott/Vysis, Downers Grove, IL) and a previously designed FISH probe for *IRF4* (BAP)[4] were used.

Potential point mutations detected by MIP-assay in *PIK3CA, FBXW7*, *ABL1*, *NOTCH1*, *STK11* and *PTEN* were also analyzed by direct sequencing using ABI PRISM 3100 Genetic Analyzer system (Applied Biosystems, Foster City, CA). Details are described in the supplementary files. Similarly, the coding exons of *TNFRSF14* (Supplementary Table 2) and Tyr641 *EZH2*[4] were also analyzed by direct sequencing in the whole series.

Clonality analysis was performed investigating the framework 1-3 regions of the immunoglobulin heavy chain (*IGH*) according to the Biomed-2 protocol.[11]

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Statistical analyses were performed using PASW Statistics software version 18 (SPSS Inc., Chicago, IL).

The study was performed in the framework of the BFM-NHL trial, for which central and local institutional review board approvals were obtained and according to the guidelines of the MMML Network Project of the Deutsche Krebshilfe (approved by the Institutional Review Board (IRB) of the Medical Faculty Kiel under 403/05).

Results and Discussion

Pediatric FL is a rare disease that differs from its adult counterpart both genetically and clinically.[3] We recently described a distinct subset of germinal center B-cell lymphomas including FL characterized by the presence of *IRF4* gene translocations, predominately affecting children and young adults.[4] In the present study, we determined genetic aberrations in a series of 18 pediatric FL cases lacking such *IRF4* translocation. Of these 18 FL, nine (50%) were classified as FL grade 3a (FL3a), six (33%) as grade 3b (FL3b), one (6%) as grade 3 unclassified and two (11%) as grade 2. Areas of grade 1 or 2 FL as a second lymphoma component were detectable in four cases diagnosed as FL3a (22%), while a simultaneous diffuse large B-cell lymphoma (DLBCL) component was noted in four (22%) cases, three diagnosed as FL3b and one as FL3a. Seven patients presented with stage I disease, five were rated as stage II and three suffered from stage III disease. Tumor localization in or dissemination to neck region was the most frequent presentation (11 of 18). Notably, the present series of pediatric FL seems to differ from those of other recently published series with regard to sites of involvement, clinical stage and presence of a DLBCL component.[12,13] This might be due to the fact that the present series predominately reflects population-based

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ascertainment for a pediatric clinical trial rather than collections at consultation centers. The patients were predominantly male (61%), with a median age of 12.1 years (range, 6-18 years). All cases evaluable for the respective analyses lacked *BCL2* breakpoints and/or *IGH-BCL2* fusion and *MYC* breakpoints by FISH. Breaks in *IGH* locus occurred in four of 15 evaluable cases whereas a *BCL6* break occurred in one of 17 cases (Supplementary Table 1).

CN determination by aCGH and/or MIP-assay was successful in 16 of the 18 FL (89%) (Supplementary Figure 1). Eight samples were analyzed on both CN platforms showing a good agreement (with seven showing the same imbalances by both platforms). Only one case (pFL12) with low quality DNA showed differences between platforms, with a chromosome 7 gain only detectable using MIP-assay. Only six of 16 evaluable pediatric FL (37%) displayed CN alterations and/or copy number neutral-loss of heterozygosity (CNN-LOH), with a mean of 6.5 aberrations per case (Figure 1A and Supplementary Tables 3 and 4). These findings contrast with adult FL, where the number of cases with chromosomal imbalances[14] and CNN-LOH[15] is higher (71.6% *vs.* 37.5%, *P*-value=0.01 and 76% *vs*. 40%, *P*-value=0.05, respectively). The most frequent imbalance in pediatric FL was 6pter-p24.3 gain including amplification (3/6 aberrant cases). Despite *IRF4* was shown by FISH to be included in the gained region (Supplementary Figure 2), no consistent expression of IRF4/MUM1 was observed in these cases (2/3 expressed IRF4/MUM1). Remarkably, three cases showed CNN-LOH in 1p36.32-p36.13 region (pFL7, pFL8, and pFL14) (Figure 1B) and another one showed a deletion of the whole 1p arm (pFL5). In comparison with three previously published pediatric FL positive for *IRF4* translocation,[16] cases positive for the

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translocation were significantly more complex in terms of CN alterations than the *IRF4* negative pediatric FL (7 alterations/case vs. 2.4 alterations/case p=0.029) (Supplementary Figure 3). Of note, *IRF4* translocation positive cases presented more frequently gains of 11q and deletions of 17p arm including *TP53* gene. Furthermore, one of the cases showed a deletion at 1pter-p35.2 with a transition mutation in the *TNFRSF14* gene (g.343C>T, T15I) (data not shown).

Both the gain in 6p as well as CNN-LOH/loss of 1p seem to be not exclusive to pediatric FL, since they have been also described in adult FL independently of the presence of t(14;18) translocation.[14] In fact, deletions and CNN-LOH of 1p36 have been described as one of the most frequent secondary genetic aberrations in adult FL[15] and are considered a significant predictor of poor overall survival.[17] The minimal region of loss/CNN-LOH in 1p36 contains the candidate gene *TNFRSF14*, encoding a member of the tumor necrosis factor receptor (TNFR) superfamily, which has been shown to be recurrently mutated in adult FL.[18,19] Sequencing of *TNFRSF14* in the four pediatric FL with 1p36 aberration revealed three to carry mutations, including one missense mutation in exon 1 (g.340C>G, S14C; pFL7), two splice donor site mutations of the exon 1 (g.370T>C and g.370T>A; pFL7 and pFL10) and one nonsense mutation in exon 5 (g.4336C>T, Q180X; pFL14). Extension of the *TNFRSF14* mutation screening to the rest of the cases revealed five non-synonymous mutations in four pediatric FL without 1p36 aberration (Supplementary Table S5). Thus, 7/17 pediatric FL contain mutations in *TNFRSF14* rendering this the most recurrent change in this disease hitherto known. In order to identify the incidence of previously described EZH2

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protein mutation (Tyr641),[20] the whole series of pediatric FL was sequenced, finding two cases mutated (pFL3 and pFL15) (Supplementary Table 3).

A synopsis of the obtained molecular profiling results revealed that all cases with breaks in *IGH* or *BCL6* also showed CN alterations and/or mutations in *TNFRSF14* or *EZH2* genes. Based on the results of the molecular analyses, two groups of FL could be distinguished: one containing the genetically aberrant pediatric FL and another one comprising the pediatric FL without any aberration (Figure 1C). The absence of genomic aberrations due to a low content of tumor cells was ruled out in all the patients (>50% tumor cell content) except in case pFL13 (20%). Nevertheless, we cannot rule out the percentage of (sub)clonal cells harboring a genetic aberration might influence the grouping.

Studying *IGH* clonality revealed that the two samples with polyclonal pattern (pFL2 and pFL11) were in the set lacking genomic aberrations. In one of the cases (pFL2) only the FR3 region was evaluable in the central *IGH* clonality analysis. Therefore, we cannot exclude that clonality could be detectable with FR1 or FR2 primers. Despite the polyclonal pattern, both cases displayed clear histomorphological features of malignancy with subtotal effacement of the underlying lymphnode structure, destruction of the normal germinal center architecture, loss of follicle mantles and abnormality enlarged in part confluent neoplastic follicles. Although there is general agreement regarding the absence of *BCL2*-rearrangement in pediatric FL at the molecular level, reported levels of BCL2 protein expression vary considerably ranging from 0% to 50%.[2,3,12,13] In our series, the majority of the cases (12/15) expressed the protein to some degree. The relatively high proportion of BCL2 expressing cases in our

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series, in comparison to previous reports, might be due to the selection criteria applied, like including cases of FL with simultaneous DLBCL component and excluding cases with *IRF4* translocations. No correlation was found between BCL2 expression and the presence of genetic aberrations.

Comparing the two groups defined by the genomic aberration status with clinical data, the group with aberrations was enriched for patients showing higher grade histology and higher stage disease patterns. E.g. advanced disease stage (>II), grade 3b or a DLBCL component occurred almost exclusively in the genetically altered group (Table 1). In contrast, lack of genetic aberrations occurred in clinical stages (I and II) and was never associated with a DLBCL component, although this was not statistically significant probably because of the low number of cases (Figure 1C). Notably, comparing cases with and without neck region tumor localization/involvement, the second group was significantly associated with the presence of genomic aberrations (P=0.03). Previous studies have shown that tonsillar pediatric FL are nearly always positive for MUM1/IRF4.[13] Nevertheless, the present series shows few cases (pFL4 and pFL 15) with tonsillar involvement, which at least in part is likely due to exclusion of the cases harboring *IRF4* translocations known to be strongly MUM1 positive.[4]

Despite these biologic differences, the overall survival was comparable between the groups with genomic aberrations and without them (Table 1 and Supplementary Table 1). This might be associated with the fact that most patients (12/16) besides the diagnostic lymph node excision received treatment with multi-agent chemotherapy. The whole series showed an excellent survival with all patients being alive at the last follow-up, independently of the biologic subgrouping, with a median follow up of 60.6 months.

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In summary, we describe the pattern of genomic imbalances in *IRF4* translocation negative pediatric FL and identify recurrent mutation of *TNFRSF14*. Moreover, we show genetic alterations to distinguish two subsets of pediatric FL: in the first subset genomic aberrations could be identified with the techniques applied and this subset is associated with higher grade and/or diffuse-large-B-cell lymphoma component and more widespread disease. The second group lacks genetic alterations detectable with the present approaches and is associated with a more limited disease. Despite the absence of genomic aberrations, these cases resembled FL by their histopathological features (Figure 2). The absence of genetic aberrations detectable with the methods used in the current study does not necessarily mean that these lesions are completely devoid of genomic aberrations. Since the histopathology of these lesions is not compatible with reactive conditions, it seems possible that a higher resolution of genomic analysis like next generation sequencing will reveal aberrations. In turn, the presence of genetic aberrations does not ultimately proof malignancy as documented e.g. in monoclonal gammopathy of undetermined significance (MGUS).[1] Indeed, it could well be that at least a subset of pediatric FL could similarly represent monoclonal follicular proliferations of undetermined significance.

Given that the patients included in this study have been treated independently of the genomic aberration status and show an excellent survival, thus we cannot comment on whether the detectability or pattern of genomic differences also translate into a different clinical outcome and whether the genetically normal appearing FL might be treated less intensively or can even be simply watched in completely resected local disease.

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Authorship and Disclosures

IMG and IS performed molecular analysis including array-CGH, analyzed data and wrote the manuscript; MS, AGO and SP performed molecular analysis; SB performed array-CGH; MB performed the CGH figure; WK, IO provided samples and performed pathology review; BB, JL, HH and LL provided samples and clinical data; RS designed the project, analyzed the data, and wrote the manuscript. Conflict of interest disclosure: RS receives speaker's honorarium and, in the framework of the MMML, probe discounts from the company Abbott/Vysis and has been supported for a test trial of arrays by the company Affymetrix. The remaining authors declare no conflict of interest.

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References

1. Swerdlow SH, Campo E, Harris N, Jaffe E, Pileri S, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press. 2008

2. Lorsbach RB, Shay-Seymore D, Moore J, Banks PM, Hasserjian RP, Sandlund JT, et al. Clinicopathologic analysis of follicular lymphoma occurring in children. Blood. 2002;99(6):1959-64.

3. Oschlies I, Salaverria I, Mahn F, Meinhardt A, Zimmermann M, Woessmann W, et al. Pediatric follicular lymphoma--a clinico-pathological study of a population-based series of patients treated within the Non-Hodgkin's Lymphoma--Berlin-Frankfurt-Munster (NHL-BFM) multicenter trials. Haematologica. 2010;95(2):253-9.

4. Salaverria I, Philipp C, Oschlies I, Kohler CW, Kreuz M, Szczepanowski M, et al. Translocations activating IRF4 identify a subtype of germinal-center-derived B-cell lymphoma affecting predominantly children and young adults. Blood. 2011;118(1):139-47.

5. Reiter A, Schrappe M, Tiemann M, Ludwig WD, Yakisan E, Zimmermann M, et al. Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: A report of the Berlin-Frankfurt-Munster Group Trial NHL-BFM 90. Blood. 1999;94(10):3294-306.

6. Woessmann W, Seidemann K, Mann G, Zimmermann M, Burkhardt B, Oschlies I, et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: a report of the BFM Group Study NHL-BFM95. Blood. 2005;105(3):948-58.

7. Zettl A, Strobel P, Wagner K, Katzenberger T, Ott G, Rosenwald A, et al. Recurrent genetic aberrations in thymoma and thymic carcinoma. The American journal of pathology. 2000;157(1):257-66.

8. Wang Y, Carlton VE, Karlin-Neumann G, Sapolsky R, Zhang L, Moorhead M, et al. High quality copy number and genotype data from FFPE samples using Molecular Inversion Probe (MIP) microarrays. BMC Med Genomics. 2009;2:8.

9. Oschlies I, Klapper W, Zimmermann M, Krams M, Wacker HH, Burkhardt B, et al. Diffuse large B-cell lymphoma in pediatric patients belongs predominantly to the germinal-center type B-cell lymphomas: a clinicopathologic analysis of cases included in the German BFM (Berlin-Frankfurt-Munster) Multicenter Trial. Blood. 2006;107(10):4047-52.

10. Ventura RA, Martin-Subero JI, Jones M, McParland J, Gesk S, Mason DY, et al. FISH analysis for the detection of lymphoma-associated chromosomal abnormalities in routine paraffin-embedded tissue. J Mol Diagn. 2006;8(2):141-51.

11. van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender FL, et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia. 2003;17(12):2257-317.

12. Louissaint A, Ackerman AM, Dias-Santagata D, Ferry JA, Hochberg EP, Huang MS, et al. Pediatric-type nodal follicular lymphoma: an indolent clonal proliferation in children and adults with high proliferation index and no BCL2 rearrangement. Blood. 2012;120(12):2395-404.

13. Liu Q, Salaverria I, Pittaluga S, Jegalian AG, Xi L, Siebert R, et al. Follicular Lymphomas in Children and Young Adults: A Comparison of the Pediatric Variant With Usual Follicular Lymphoma. Am J Surg Pathol. 2013; 37(3):333-43.

14. Leich E, Salaverria I, Bea S, Zettl A, Wright G, Moreno V, et al. Follicular lymphomas with and without translocation t(14;18) differ in gene expression profiles and genetic alterations. Blood. 2009;114(4):826-34.

15. Cheung KJ, Delaney A, Ben-Neriah S, Schein J, Lee T, Shah SP, et al. High resolution analysis of follicular lymphoma genomes reveals somatic recurrent sites of copy-neutral loss of heterozygosity and copy number alterations that target single genes. Genes Chromosomes Cancer. 2010;49(8):669-81.

16. Salaverria I, Martin-Guerrero I, Burkhardt B, Kreuz M, Zenz T, Oschlies I, et al. High Resolution Copy Number Analysis of IRF*4 Tra*nslocation-positive Diffuse Large B- and Follicular Lymphomas. Genes Chromosomes Cancer. 2013;52(2):150-5.

17. Cheung KJ, Shah SP, Steidl C, Johnson N, Relander T, Telenius A, et al. Genome-wide profiling of follicular lymphoma by array comparative genomic hybridization reveals prognostically significant DNA copy number imbalances. Blood. 2009;113(1):137-48.

18. Cheung KJ, Johnson NA, Affleck JG, Severson T, Steidl C, Ben-Neriah S, et al. Acquired TNFRSF14 mutations in follicular lymphoma are associated with worse prognosis. Cancer Res. 2010;70(22):9166-74.

19. Launay E, Pangault C, Bertrand P, Jardin F, Lamy T, Tilly H, et al. High rate of TNFRSF14 gene alterations related to 1p36 region in de novo follicular lymphoma and impact on prognosis. Leukemia. 2012;26(3):559-62.

20. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42(2):181-5.

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Table 1. Comparison of clinical and biological characteristics of pediatric follicular lymphoma (FL) with genomic aberrations *versus* pediatric FL without aberrations

Clinical and biological characteristics	Ped FL with genomic aberrations	Ped FL without genomic aberrations	*P*-value
Gender ratio (M:F)	1.2:1	2.5:1	0.64
Age (median)	12.1	12	0.97
Grade FL			
1-2	1	1	0.31
3a	4[a]	5[b]	
3b	5	1	
grade 3 unknown	1	-	
Stage			
I	2	5	0.11
II	3	2	
III	3	0	
nd	3	-	
DLBCL component			
No	7	7	0.12
Yes	4[c]	0	
Localization			
Neck region	4	7	**0.03**
Other regions	4	0	
Both	3	0	
Remnants of reactive B-follicles			
No	5	2	0.38
Yes	5	5	
nd	1	-	
Marginal zone differentiation			
No	9	4	0.33
Yes	2	3	
Ki67 (%)	55[d]	54[e]	0.99
Tumor cell content (%)	76.4	67.9	0.32
Median follow-up (months)	73.8[e]	43.6	0.21

nd, not determined; DLBCL; diffuse large B-cell Lymphoma; M, male; F, female
[a]One case with FL1/2 as a second lymphoma component
[b]Three cases with FL1/2 as a second lymphoma component
[c]Case pFL8 present starry sky pattern- Burkitt like pattern
[d]Data not available in one case
[e]Data not available in two cases

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Figure legend

Figure 1. (A) Copy number (CN) profiles of 16 pediatric FL. In the X-axis the chromosomes are represented horizontally from 1 to 22, in the Y-axis the percentage of cases showing the CN alterations. Gains are represented in the positive Y-axis and colored in yellow, whereas losses are represented in the negative Y-axis in blue. The most frequent CN alteration was gain/amplification of 6pter-p24.3. **(B)** Molecular Inverse Probe (MIP)-assay profiles showing CN neutral loss of heterozygosity (CNN-LOH) at 1p36 in the pFL7, pFL10 and pFL14 cases. Allelic events are displayed along the X axis (from pter to qter). Germline homozygosity (e.g. AA, BB alleles) at a given SNP results in calls at the 0 and 1 levels, respectively, germline heterozygosity (AB-alleles) in calls around 0.5 (Y-Axis: 0-1). CNN-LOH in the tumor leads to loss of calls around 0.5 and to the presence of allelic imbalance calls derived from a sum of heterozygous normal cell (AB) and homozygous tumor cell (AA or BB) calls for a given locus resulting in values between 0-0.5 or 0.5-1 depending on the amount of cells carrying the aberration. Thus, in the areas when only contribution from one parent (LOH/CNN-LOH), two bands should we expected (0% and 100%->0 and 1.0. Y Axis). In the three cases (pFL7, pFL10, pFL14), the probes did not reach such thresholds because alterations are detected to be in mosaicism (not germline alterations). **(C)** Summary data of the FISH, CN, CNN-LOH, 1p36, *TNFRSF14*, *EZH2* mutation, *IGH* clonality, BCL2 expression analyses and DLBCL component for the 18 pediatric FL patients. FISH was considered positive when splits in the *BCL2*, *BCL6*, *MYC*, *IRF4* and *IGH* genes were detected; CN, when chromosomal imbalances were found by MIP-assay or aCGH; 1p36, when CNN-LOH (detected by MIP-assay) or deletions (by MIP-assay and/or aCGH) were observed at that region; *TNFRSF14* and *EZH2*, when

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mutations were found by direct sequencing; *IGH* clonality was positive when clonal *IGH* chain gene rearrangement was detected by PCR; BCL2 expression was positive when more than 25% of the cells were positive. In pFL2 only the region FR3 was evaluable in the *IGH* PCR reaction. In pFL18 *IGH* monoclonality was based on an analysis performed in an outside laboratory. CNN-LOH could not be determined in cases 1, 2, 12, 16, 17, and 18.

Figure 2: A-H: Histomorphological features of case pFL11 (FL3a) **(A-D)** without aberrant genotype and case pFL14 (FL3a) **(E-H)** with aberrant genotype: in both cases an infiltration of the lymphnode by enlarged abnormal follicles as well as remnants of normal reactive follicles (indicated by arrows) are seen in the low magnification (**A,E,** H&E x 25). The neoplastic follicles lack a clear starry sky pattern as well as the demarcation by a mantle zone (**B,F,** H&E x 100). The follicles consist of centroblasts and centrocytes (**C,G,** H&E x 1000). The lack of zonation is seen with immunohistochemistry for the proliferation marker Ki67 (**D, H,** Ki67 x 100), nevertheless a hot spot of proliferation can be observed in **H**.

Figure 1

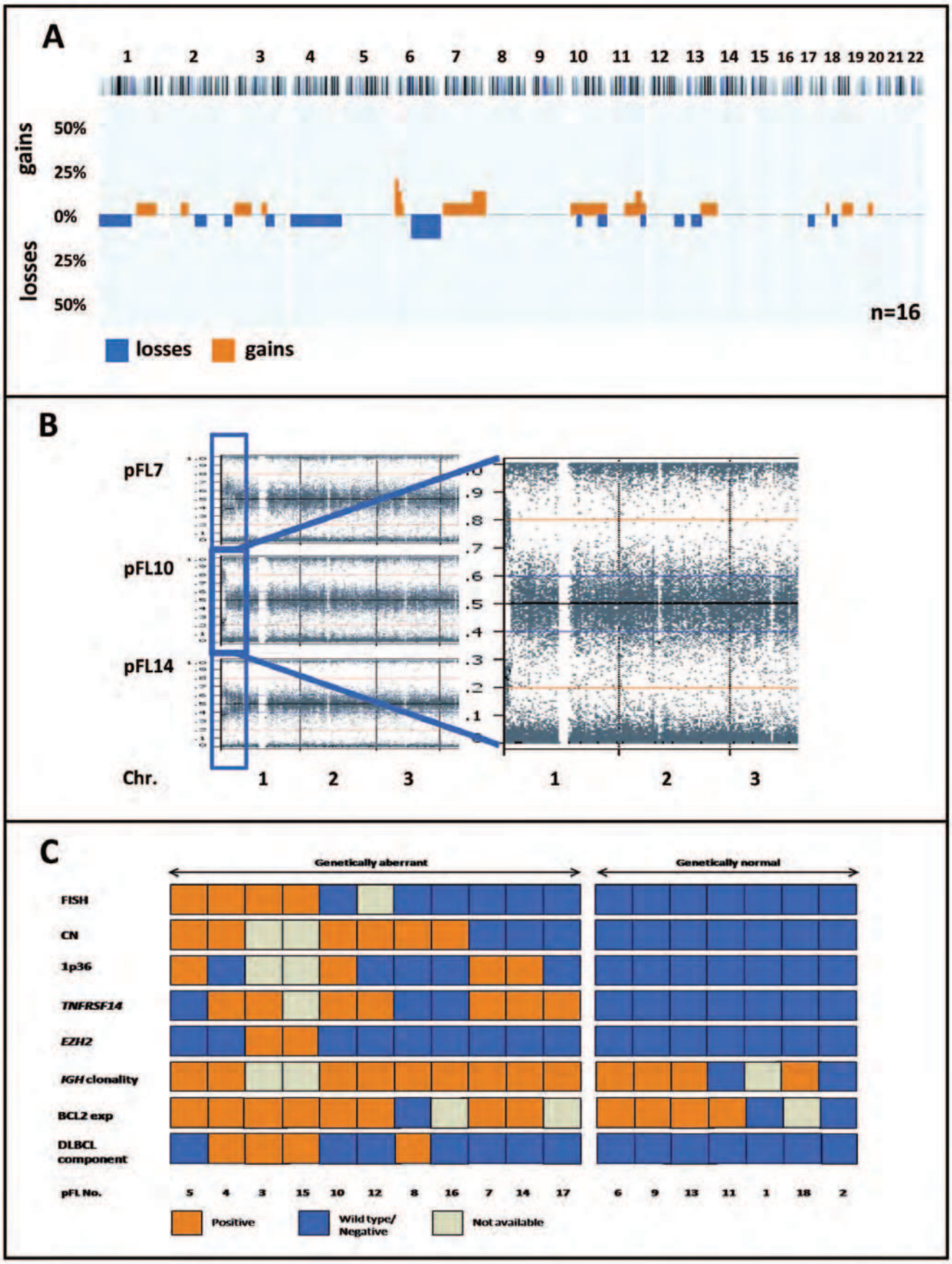
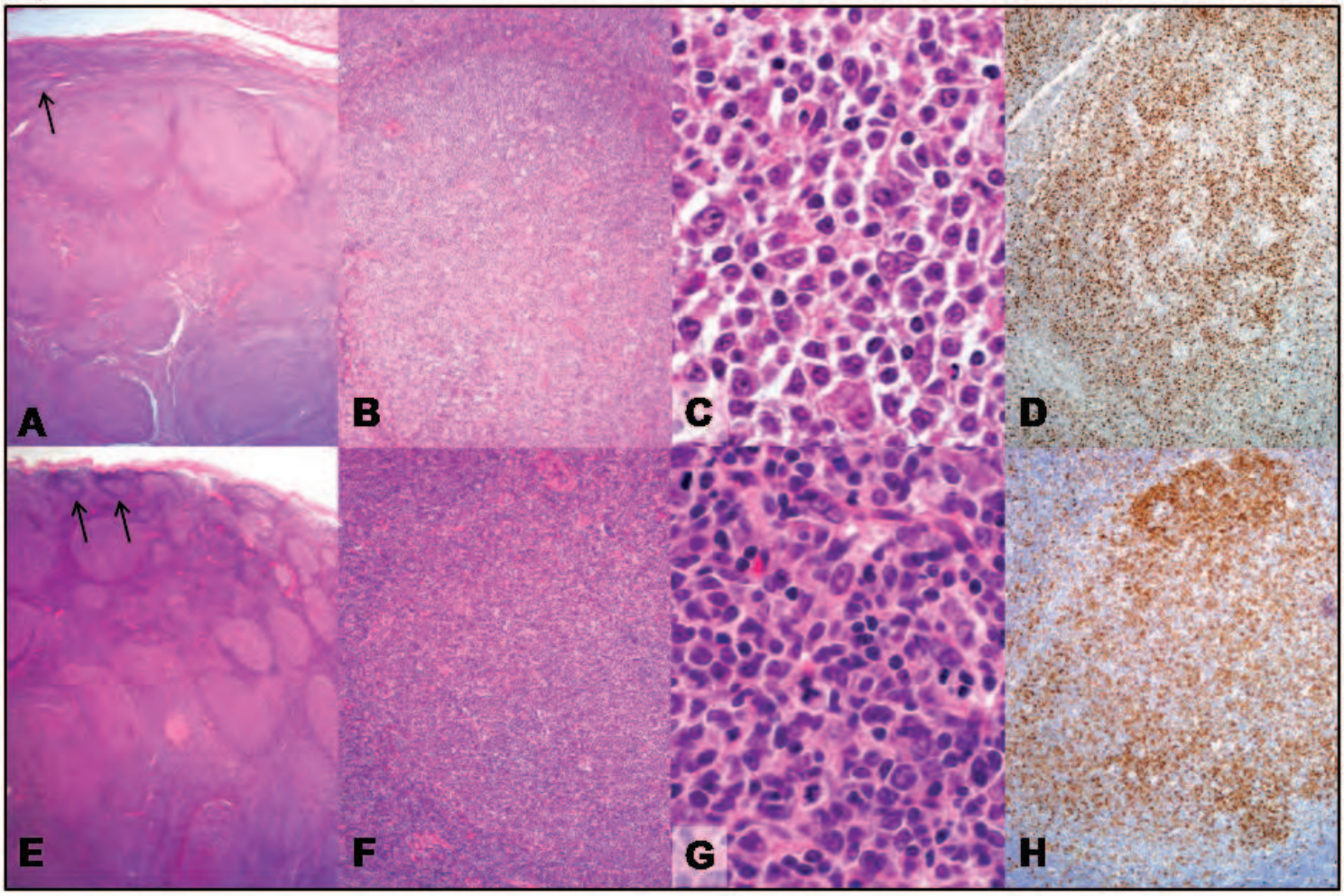

A
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
gains
losses
50%
25%
0%
25%
50%
n=16
losses
gains
B
pFL7
pFL10
pFL14
Chr.
1 2 3
1 2 3
C
Genetically aberrant
Genetically normal
FISH
CN
1p36
TNFRSF14
EZH2
IGH clonality
BCL2 exp
DLBCL component
pFL No.
5 4 3 15 10 12 8 16 7 14 17
6 9 13 11 1 18 2
Positive
Wild type/ Negative
Not available

Figure 2



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Supplementary information

Recurrent loss of heterozygosity in 1p36 associated with *TNFRSF14* mutations in *IRF4* translocation negative pediatric follicular lymphomas

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I. Methods

Determination of tumor content

The tumor cell content was estimated by visual inspection on full slides of the respective lymphoma block used for the molecular analyses with help of the immunohistochemical stains for CD20 and at least one T-cell marker. Confusion with reactive B-cell-areas should not have caused significant wrong estimations as usually the lymphoma was more or less completely effacing the preexisting B-cell areas and as significant areas with small reactive follicles can be recognized cytologically even within the CD20-staining.

The OncoScan FFPE Express assay copy number and somatic mutation determination

DNAs from formalin-fixed paraffin-embedded material were hybridized on the MIP-assay using Oncoscan FFPE Express custom service (Affymetrix, Santa Clara, USA). Copy number determination of MIP-assay has been previously described.[1] Briefly, MIP probes are oligonucleotides in which the two end sequences are complementary to two adjacent genomic sequences; these two ends anneal to the genomic DNA in an inverted fashion with a single base between them (generally the site of a single nucleotide polymorphism; SNP). In copy number analysis, genomic DNA is hybridized to the MIP probe and the reaction split into two separate tubes containing paired nucleotide mixes.[2] Amplification and ligation allows circularization of the MIP probe. The probes are then amplified, labeled, detected and quantified by hybridization to tag microarrays. Gains and losses were defined by the use of a trial version of Nexus 6.0 beta Discovery Edition (Biodiscovery, El Segundo, USA) that integrates the genomic identification of significant targets in Cancer (GISTIC) algorithm for identifying significant regions of common genomic aberrations as well as the Allele specific Copy number Analysis of tumors (ASCAT) algorithm for addressing sample aneuploidy and mosaicism. The average resolution was of ~9 Kb/probe for whole genome backbone and ~3 Kb/probe for intragenic regions.

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OncoScan FFPE Express also analyzes ~300 somatic mutations in relevant cancer genes. These files are generated from the raw CEL files as part of the primary data analysis process using Affymetrix software. Each sample has a score for each assay (each assay interrogates one previously described mutation). According to manufacturer's instructions a score of 9 or higher indicates that an assay is a valid somatic mutation for that sample. According to this criterion, PIK3CA_pR88Q_c263G_A, FBXW7_pR278X_c832C_T, ABL1_pF359V_c1075T_G, NOTCH1_pL1586P_c4757T_C, PTEN_p_c165_minus_2A_C and PTEN_pQ110X_c328C_T mutations were found. No mutation detected by MIP assay was confirmed by Sanger sequencing.

Array-CGH

Microarray analysis has been performed using the Human Genome CGH Microarray 244A platform (Agilent Technologies, Santa Clara, USA). Experimental procedures were performed according to the manufacturer's instructions. The array was scanned with the DNA microarray scanner (Agilent Technologies) at a resolution of 5µm/pixel. Signal intensities from the generated images were measured and evaluated with the Feature Extraction v10.7.3.1 and Agilent Genomic Workbench Standard Edition 6.5.0.58 software packages (Agilent Technologies). The average genome-wide resolution was of 0.65 Mb.

Mutations

Non-synonymous mutations were tested for their functional consequences *in silico* by using different aminoacid substitution prediction algorithms, including SIFT (Sorting Intolerant From Tolerant) and PolyPhen-2 (Polymorphism Phenotyping).

Statistical analyses

Statistical analyses were performed using PASW Statistics software version 18 (SPSS Inc., Chicago, USA). Chi-Square or Fisher´s exact tests were used to determine the significance of any differences between the clinical variables in samples with genomic aberrations *vs.* wild

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type. Overall survival (OS) was defined as the time from diagnosis to date of most recent follow-up. All statistical tests were considered significant at $P \leq 0.05$.

Informed consent

The study was performed in the framework of the BFM-NHL trial, for which central and local institutional review board approvals were obtained and according to the guidelines of the MMML Network Project of the Deutsche Krebshilfe (approved by the IRB of the Medical Faculty Kiel under 403/05).

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I. Supplementary tables

Supplementary Table S1. Clinical, pathological and FISH data in pediatric FL

ID	Diag	2nd lymphoma component (%)	Age, sex	Tumor cells %	Stage	Loc	BCL2 exp^	Remnants of reactive B-follicles	Marginal zone diff	Treatment Protocol	Outcome	Follow-up (m)	*BCL2/ t(14;18)*	*BCL6*	*MYC*	*IRF4*	*IGH*	*Ref Oschlies et al*[3]
pFL1	FL3a	-	8,m	70	II	LN (c)	no	yes	yes	NHL-BFM 90	LFU in CR	114	wt	wt	na	wt	wt	4
pFL2	FL3a	FL 1	11,m	65	I	LN (c)	no	yes	yes	NHL-BFM 90	LFU in CR	84	wt	wt	wt	wt	wt	6
pFL3	FL3b	DLBCL (90) [&]	15,m	65	III	LN (ab), intestine	yes (partial)	yes	no	NHL-BFM 95	LFU in CR	156	wt	wt	na	na	split	10
pFL4	FL3b	DLBCL (50)[$]	8,m	90	II	T, LN (c)	yes	yes	no	NHL-BFM 90	LFU in CR	120	wt	wt	wt	na	split	13
pFL5	FL3b	-	10,f	95	III	LN (c, ax, med, ab, ing)	yes	no	no	NHL-BFM 95	Relapse DD second malignancy**	112	wt	split	wt	wt[#]	split	16
pFL6	FL3a	FL 2	16,m	90	I	LN (c)	yes	no	no	NHL-BFM 95	LFU in CR	17	wt	wt	wt	wt	na	18
pFL7	FL3a	-	17,m	80	I	LN (c)	yes (partial)	no	yes	B-NHL BFM 04	Alive in CR	59	wt	wt	wt	wt	wt	21
pFL8[†]	FL3b	DLBCL (30)	6,f	75	II	LN (c)	no	no	no	Ritux + B-NHL BFM 04	Alive in CR	24	wt	wt	wt	wt	wt	23
pFL9	FL3a	FL2	15,f	75	I	LN (c)	yes	yes	no	B-NHL BFM 04	Alive in CR	24	wt	wt	wt	wt	wt	25
pFL10	FL3a	-	6,m	70	na	LN (femoral)	yes	na	yes	R-CHOP	LFU in CR	36	wt	wt	wt	wt[#]	wt	-
pFL11	FL3a	-	10,m	75	II	LN (c)	yes (weak)	yes	yes	B-NHL BFM 04	Alive in CR	19	wt	wt	wt	wt	wt	-
pFL12	FL2	-	17,f	80	na	LN (c, med)	yes	no	no	na	na	na	na	na	na	na	na	-
pFL13	FL3b	-	6,f	20	I	LN (retroauricular)	yes	yes (majority)	no	complete extirpation, w&w	Alive in CR	11	wt	wt	wt	wt	wt	-
pFL14	FL3a	FL1 and 2	18,m	80	I	LN (ing)	yes	yes	no	complete extirpation, w&w	Alive in CR	16	wt	wt	wt	wt	wt	-
pFL15	FL3a	DLBCL (15)	9,f	90	III	T, LN (c,ax,ab), liver, intestine	yes	yes (in tonsil)	no	NHL-BFM 95	LFU in CR	102	wt	wt	wt	wt	split	5
pFL16	FL3b	-	18,f	65	II	LN (ing)	na	no	no	Ritux	LFU in CR	39	wt	wt	wt	wt[#]	wt	-
pFL17	FL3*	-	9,m	50	na	LN (c)	na	yes	no	na	na	na	wt	wt	wt	wt	na	-
pFL18	FL2	-	18,m	80	I	LN (c)	na	no	no	complete extirpation, w&w	Alive in CR	36	wt	wt	wt	wt	wt	-

Abbreviations: Diag, diagnosis; Loc, localization; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; m, male; f, female; LN, lymph nodes; diff, differentiation; DD, differential diagnosis; T, tonsil; c, cervical including nuchal and submental; ing, inguinal; med, mediastinal; ax, axillary; ab, abdominal; wt, wild type; na, not available; FU, follow-up; LFU, lost to follow-up; CR, complete remission; w&w, watch and wait; m, month; Ref, reference

* Diagnosis as FL3a or FL3b was not available; [#] Multiple *IRF4* copies were detected by FISH; [†] The case pFL8 presents starry sky pattern- Burkitt like pattern; ** Nijmegen-breakage syndrome; differentiation between relapse and second malignancy not possible (no molecular genetics); another relapse DD second malignancy (no molecular genetics) 8 years after first lymphoma; alive; [&] The case pFL3 shows the diffuse growth in 90% of the biopsy as this is an infiltration of the intestinal wall. A secondary colonization of preexisting follicles of the Payer Plaques cannot be ruled out. Nevertheless, the histopathology nor an additional staining for FDC-networks does not allow to differentiate in this case between secondary involvement and focal true follicular growth. [$] The architecture and extension of the follicular component did not resemble secondary colonization of preexisting follicles; ^BCL2 expression data of ten cases were previously published by Oschlies et al, 2010[3]

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Supplementary Table S2. Primer information for mutational analyses.

Gene	Primer	Nucleotide sequence (5´-3´)	PCR product (bp)
TNFRSF14	TNFRSF14_ex1_F	TCCTCTGCTGGAGTTCATCC	209
	TNFRSF14_ex1_R	CATGGGGAAGAGATCTGTGG	
	TNFRSF14_ex2_F	ATCTCCCAATGCCTGTCCT	202
	TNFRSF14_ex2_R	AGAAGGGGGCAAGAGTGTCT	
	TNFRSF14_ex3_F	TAGCTGGTGTCTCCCTGCTT	250
	TNFRSF14_ex3_R	GGCTGTGCTGGCCTCTTAC	
	TNFRSF14_ex4_F	TCCACGTACCCCTCTCAGC	228
	TNFRSF14_ex4_R	GAAATGGGAGGGGTGTCC	
	TNFRSF14_ex5_F	CCTCTGTCCGTCCCTCTCTT	218
	TNFRSF14_ex5_R	ACCTTCAAGCCTTTCTGCTG	
	TNFRSF14_ex6_F	CTCCCTGAGGCTGAGTGAAC	277
	TNFRSF14_ex6_R	GGTGACAGAGCTCCAAGAGG	
	TNFRSF14_ex7_F	CTGTGTCCCCTGATCAGACA	204
	TNFRSF14_ex7_R	CAGGACCCTCAGAGAACTGG	
	TNFRSF14_ex8_F	AAAATGAACCCGAGAACCTG	267
	TNFRSF14_ex8_R	AGGTGGACAGCCTCTTTCAG	
ABL1	ABL1_F359V_F	GCTGTACATGGCCACTCAGAT	224
	ABL1_F359V_R	GAGCCTAGTGTTTGCCTTTGTT	
NOTCH1	NOTCH1_L1586P_F	ACCAGTACTGCAAGGACCACTT	220
	NOTCH1_L1586P_R	AAGACCACGTTGGTGTGCAG	
STK11	STK11_Q170X_F	CCGCAGGTACTTCTGTCAGC	128
	STK11_Q170X_R	CCAGGTCGGAGATTTTGAGG	
PIK3CA	PIK3CA_R88Q_F	GCCTCCGTGAGGCTACATTA	233
	PIK3CA_R88Q_R	GAGGATCTTTTCTTCACGGTTG	
FBXW7	FBXW7_R278X_F	ATAGAGCTGGAGTGGACCAGAG	212
	FBXW7_R278X_R	TGTTTAAAGGTGGTAGCTGTTGAG	
PTEN	PTEN_p_c165_F	AAATCTGTCTTTTGGTTTTTCTTG	274
	PTEN_p_c165_R	AATCGGTTTAGGAATACAATTCTG	
	PTEN_pQ110X_F	TAACCCACCACAGCTAGAACTT	289
	PTEN_pQ110X_R	GAAACCCAAAATCTGTTTTCCA	

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Supplementary Table S3. Genetic alterations of pediatric follicular lymphomas.

ID	No. CN alt/ CNN-LOH	CN gains	CN losses	CNN-LOH	*TNFRSF14* status	*EZH2* status	Reference Oschlies et al.[3]
pFL1	0	no	no	na	wt	wt	4
pFL2	0	no	no	na	wt	wt	6
pFL3	na	na	na	na	P16S	c.1922 A>T	10
pFL4	7	11q22.1-q25, 17q25.1-q25.3, Xpter-q28	4, 6q11.1-qter, 17p13.3-q11.1, Xq28-qter	no	T272I/G232S	wt	13
pFL5	20	1q21.1-q41, 2p16.2-p12, 3pter-p14.2, 3q13.11-q13.32, **6pter-p22.1,** 13q21.1-qter, 18q12.3-qter, 19q13.31-qter, Xq21.1-q22.2, Xq26.3-qter	1pter-p12, 2q12.1-q22.3, 2q35-qter, 3q13.32-q24, 6q11.1-qter,10p12.1-p11.1, 10q24.32-qter,12q23.1-qter, 13q11-q21.1, 18pter-q11.1	no	wt	wt	16
pFL6	0	no	no	no	wt	wt	18
pFL7	1	no	no	1p36.2-p34.1	S14C/ g.370T>C	wt	21
pFL8	5	10, 11q12.1-q23.3, **11q23.3**	11q23.3-q25	17q12-q21.2	wt	wt	23
pFL9	0	no	no	no	wt	wt	25
pFL10	3	**6pter-p22.3**, 7q31.1-q36.3	no	1pter-p36.13	g.370T>A	wt	-
pFL11	0	no	no	no	wt	wt	-
pFL12	1	7	no	na	P9T	wt	-
pFL13	0	no	no	no	wt	wt	-
pFL14	1	no	no	1pter-p36.13	Q180X	wt	-
pFL15	na	nd	nd	na	nd	c.1922 A>T	5
pFL16	1	6pter-p24.3	no	na	wt	wt	-
pFL17	0	no	no	na	V267M	wt	-
pFL18	0	no	no	na	wt	wt	-

Abbreviations: CN alt, copy number alterations; CNN-LOH, copy number neutral loss of heterozygosity; nd, not done; na, not available; wt, wild type
Notes: Regions in bold correspond to copy number gains of more than two copies /amplifications.
The table does not include "silent" mutations or polymorphisms.

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Supplementary Table S4. Whole copy number data of pediatric follicular lymphomas.

Case	CN status	chr	start	end	Size (Mb)
ONCOSCAN					
pFL1					
	no aberrations				
pFL2					
	no aberrations				
pFL4					
	High Gain	**11q22.1-q25**	99,634,634	134,324,142	34,69
	Gain	17q25.1-q25.3	69,229,399	78,723,423	9,49
	Gain	Xpter-q28	0	146,983,016	146,93
	Loss	Xq28-qter	146,983,017-	154,664,758	7,68
	Loss	4	0	191,273,063	191,27
	Loss	6q11.1-qter	61,941,918-	170,899,992	108,96
	Loss	17pter-q11.1	0	22,221,149	22,22
pFL5					
	High Gain	**1q21.1-q41**	143,599,182	215,218,824	71,62
	Gain	2p16.2-p12	53,021,912	76,446,518	23,42
	Gain	3pter-p14.2	0	61,085,250	61,09
	Gain	3q13.11-q13.32	105,664,486	119,311,190	13,65
	Gain	6pter-p22.1	0	29,392,572	29,39
	Gain	13q21.1-qter	54,580,422	114,068,620	59,49
	Gain	18q12.3-qter	40,314,818	76,117,153	35,80
	Gain	19q13.31-qter	49,926,901	63,728,509	13,80
	Gain	Xq21.1-q22.2	81,184,537	102,751,070	21,57
	Gain	Xq26.3-qter	136,020,499	154,913,754	18,89
	Loss	1pter-p12	0	119,845,934	119,85
	Loss	2q12.1-q22.3	104,461,080	145,137,592	40,68
	Loss	2q35-qter	218,893,446	242,634,600	23,74
	Loss	3q13.32-q24	119,441,159	146,084,725	26,64
	Loss	6q11.1-qter	61,871,980	170,821,724	108,95
	Loss	10p12.1-p11.1	24,340,995	38,892,677	14,55
	Loss	10q24.32 - qter	103,978,381	135,198,353	31,22
	Loss	12q23.1 - qter	99,413,038	132,349,534	32,94
	Loss	13q11-q21.1	18,069,540	54,580,422	36,51
	Loss	18pter-q11.1	49,588	16,116,010	16,07
pFL14					
	CNN-LOH	1pter-p36.13	0	18,214,258	18,21
ONCOSCAN/AGILENT244K					
pFL6					
	no alterations				
pFL7					
	CNN-LOH	1p36.32-p34.1	5,151,053	45,818,122	40,67
pFL8					
	Gain	10	0	135,374,737	135,37
	Gain	11q12.1-q23.3	58,686,057	117,196,931	58,51
	High Gain	**11q23.3**	117,207,811	118,931,293	1,72
	Loss	11q23.3-qter	118936,999	134,431,956	15,49
	CNN-LOH	17q21.31 - qter	40,729,078	78,774,742	38,05

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pFL09					
	no alterations				
pFL10					
	High Gain	**6pter-p22.3**	0	20,530,644	20,53
	Gain	7q31.1-qter	113,399,531	158,717,957	45,32
	CNN-LOH	1pter-p36.13	0	17,582,810	17,58
pFL11					
	no alterations				
pFL12					
	Gain	7	0	158,821,424	158,82
pFL13					
	no alterations				
AGILENT244K					
pFL16					
	Gain	6pter-p24.3	181,550	9,878,354	9,7
pFL17					
	no alterations				
pFL18					
	no alterations				

Regions in bold correspond to copy number gains of more than two copies/ amplifications

Numbering according to the Human Genome hg18/NCBI build 36.1 assembly

CN: copy number; CNN-LOH, copy number neutral loss of heterozygosity

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Supplementary Table S5. *TNFRSF14* mutational analysis of pediatric follicular lymphomas.

Case	Diagnosis	1p36 aberration	Exon	Position[&]	Ref nt	Obs nt	Mutation type	Effect	SIFT prediction (score*)	Polyphen prediction
pFL7	PedFL	yes	1	2486274	C	G	missense	S14C	tolerant (0.05)	benign
			1	2486244	T	C	splice site	-	-	-
pFL10	PedFL	yes	1	2486244	T	A	splice site	-	-	-
pFL14	PedFL	yes	5	2482278	C	T	nonsense	Q180X		
pFL3	PedFL	no	1	2486269	C	T	missense	P16S	tolerant (0.34)	probably damaging
pFL4	PedFL	no	6	2481164	G	A	missense	G232S	tolerant (0.51)	benign
			8	2479743	C	T	missense	T272I	tolerant (0.05)	possibly damaging
pFL12	PedFL	no	1	2486290	C	A	missense	P9T	tolerant (0.6)	benign
pFL17	PedFL	no	8	2479759	G	A	missense	V267M	tolerant (0.14)	benign

PedFL: pediatric follicular lymphoma SIFT: Sorting Intolerant From Tolerant predictor; nt: nucleotide
*Treshold for intolerance is 0.05
[&]Numbering according to the Human Genome hg18/NCBI build 36.1

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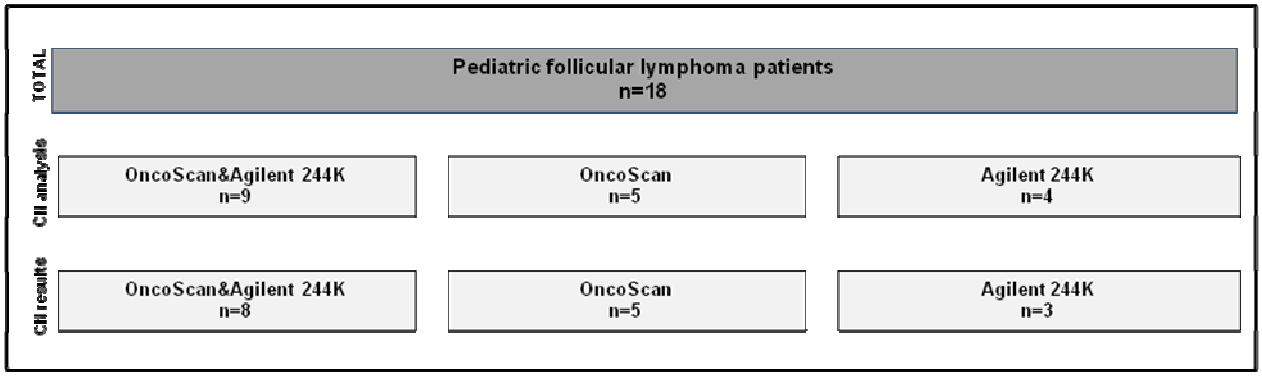
Supplementary Figures

Supplementary Figure S1. Global series included in the copy number analysis



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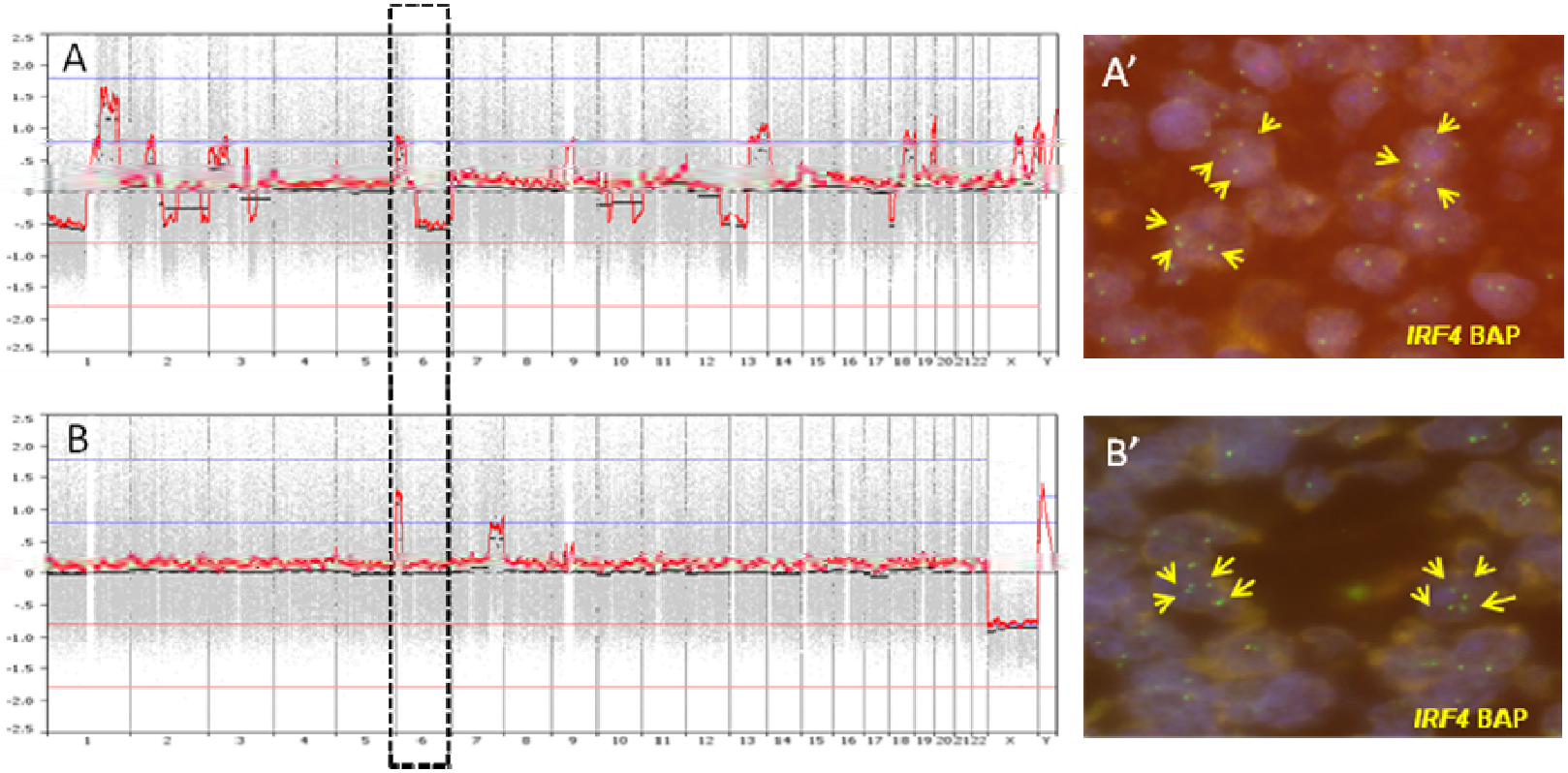
Supplementary Figure S2. (A, B) Gain and amplification of 6p arm detected by MIP-assay in cases pFL5 and pFL10, respectively. A' and B' show FISH validation of the 6p25 gain/amplification, using an *IRF4* break apart probe consisting in PAC-clone RP3-416J7 labeled in orange and BAC-clones RP5-1077H22 and RP5-856G1 labeled in green.



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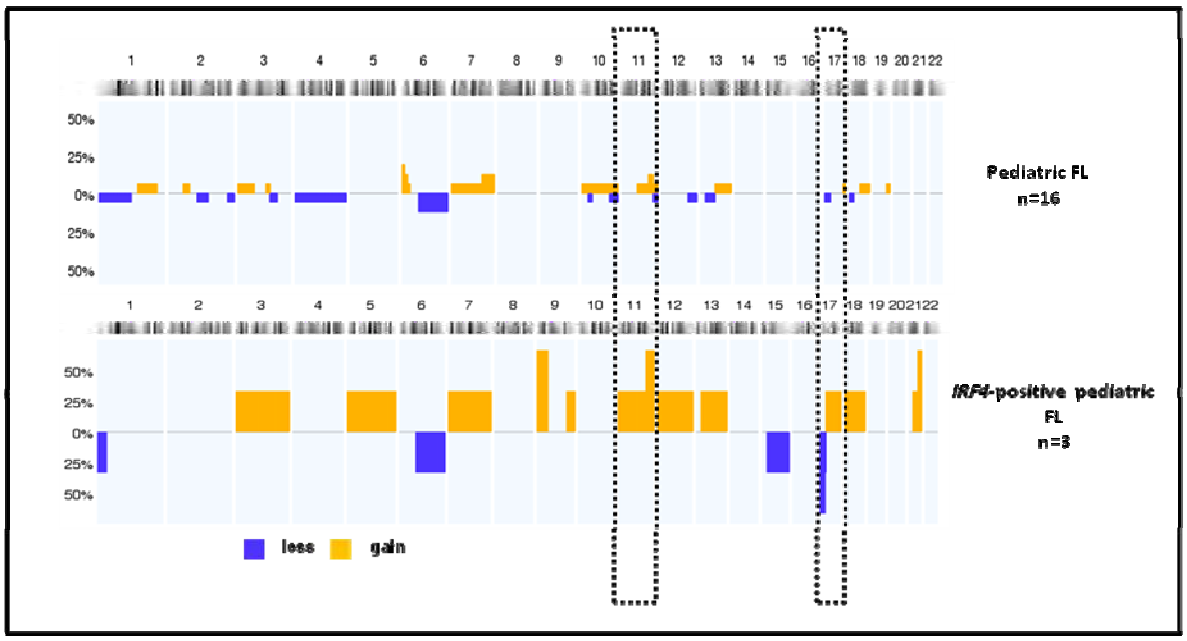
Supplementary Figure S3. Comparison of chromosomal imbalances between pediatric FL and previously published pediatric FL positive for IRF4-translocation.[4]



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Supplementary references

1. Wang Y, Carlton VE, Karlin-Neumann G, Sapolsky R, Zhang L, Moorhead M, et al. High quality copy number and genotype data from FFPE samples using Molecular Inversion Probe (MIP) microarrays. BMC Med Genomics. 2009;2:8.
2. Wang Y, Moorhead M, Karlin-Neumann G, Wang NJ, Ireland J, Lin S, et al. Analysis of molecular inversion probe performance for allele copy number determination. Genome Biol 2007;8:R246.
3. Oschlies I, Salaverria I, Mahn F, Meinhardt A, Zimmermann M, Woessmann W, et al. Pediatric follicular lymphoma--a clinico-pathological study of a population-based series of patients treated within the Non-Hodgkin's Lymphoma--Berlin-Frankfurt-Munster (NHL-BFM) multicenter trials. Haematologica. 2010;95:253-9.
4. Salaverria I, Martin-Guerrero I, Burkhardt B, Kreuz M, Zenz T, Oschlies I, et al. High Resolution Copy Number Analysis of IRF4 Translocation-positive Diffuse Large B- and Follicular Lymphomas. Genes Chromosomes Cancer. 2013;52(2):150-5.